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Efficacy of certain plant extracts on controlling bean rust

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Abstract

The water extracts of basil, henna, lemongrass, marjoram, and thyme were used at three concentrations (S/2, S/4, and S/8) to study their impact on *Uromyces appendiculatus* (Pers. ex Pers.), the causative agent of bean rust *in vitro* and at S/2 concentration *in vivo*, in the presence of the fungicide Domark 10 EC at 0.5 ml/ L as check. *In vitro*, all treatments reduced the germination percentage, and the inhibition increased with concentration. Domark emerged as the most potent agent, inhibiting spore germination by 91.9%, followed by lemongrass with a germination inhibition rate of 76.0% at S/2 concentration. *In vivo* evaluations conducted in Khatatba, Menoufia Governorate, Egypt during the agricultural seasons 2022-2023 and 2023-2024 underscored the substantial benefits of these treatments not only in mitigating the frequency and severity of *U. appendiculatus* infections but also in a significant increase in crop yield and an elevation in total carbohydrates, protein content, and phenolic enzyme activities in bean plants, including peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase. Among the tested extracts, lemongrass outperformed others, followed closely by marjoram, while basil ranked the lowest in efficacy. These findings underscore the potential of both chemical and natural treatments in managing *Uromyces appendiculatus*. The superior performance of lemongrass and marjoram highlights their relevance in integrated disease management strategies, providing valuable options for enhancing nutritional quality and bean yield.
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Keywords: bean, plant extracts, *Uromyces appendiculatus*.

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1. Introduction

Beans (*Phaseolus vulgaris* L.) are considered one of the most important food legume crops in Egypt for both domestic consumption and export. The high nutritional value of beans, including vitamins, protein, carbohydrates, and other nutrients, may explain their economic importance on a global scale (Soliman *et al*., 2013; Lizarazo *et al*., 2015). Additionally, they enhance soil fertility by fixing nitrogen. Beans are vulnerable to various types of bacteria, fungi, viruses, and nematodes, as well as physiological issues (Liebenberg and Pretorius, 2010). Bean rust is recognized as one of the most destructive diseases affecting agricultural yield (El-Fawy *et al*., 2021), particularly in Egypt's northern and central Delta regions, as well as in other countries. The ability of the sexual stage to produce variety was emphasized in the life cycle description of the rust pathogen, *Uromyces appendiculatus* (Pers. ex Pers.) Unger (Omara *et al*., 2022). Fungicides can sometimes be effective in managing plant diseases. However, improper use of fungicides often leads to environmental contamination, global risks, and the development of resistance to the diseases they aim to control (Hahn, 2014). Various plant extracts may provide an alternative to chemical management of plant pathogens. Research shows that several plant species contain antifungal compounds and have demonstrated higher increases in the number of bean plant pods compared to the control (Okemo *et al*., 2003). Therefore, it is important to utilize safe and effective alternative methods for managing this disease or at least to validate their use to address these challenges. Many fungal plant infections can be managed through biological control, which has emerged as a significant agricultural biotechnology strategy in recent years (Ahmed and Shaheen, 2016). The use of pumpkin, sage, and sesame essential oils led to a significant decrease in the severity of bean rust disease infection while also improving crop attributes and yield components, such as plant height, number of pods per plant, and 100-seed weight. Additionally, there were increases in total phenolic content and enzyme activity. Notably, the application of sage oil resulted in the most substantial increase compared to untreated plants (Hancock *et al*., 2015). The effectiveness of enzymes and plant extracts in activating various biochemical defense responses in plants against bean rust was evaluated using GC-MS to detect the presence of numerous physiologically active components, including fatty acids, esters, alcohols, phenols, antioxidants, carotenoids, enzymes, and steroids (El Fawy *et al*., 2023; Zyton and Ahmed, 2016). Spraying bean plants with the tested biotic and abiotic stimulants and fungicides, such as polymyxa, *Trichoderma harzianum*, *T. viride*, ascorbic acid, Bion, potassium citrate, and salicylic acid, along with the fungicides Domark 10 EC and Tilt EC 25%, reduced the severity of bean rust disease and enhanced yield parameters, chemical components, enzyme activity, and overall yield (Basiony *et al*., 2023). The aim of this study was to identify alternative sources of harmful chemicals in the food chain, such as plant extracts, and evaluate their effectiveness in preventing bean rust, as well as their impact on crop components, total phenolic content, and the activity of certain oxidative enzymes in treated plants.

2. Materials and methods

2.1 Plant extracts

Plants were collected from markets and their aboveground parts were washed with tap water, air-dried, cut into small pieces, and frozen. Aqueous extracts of basil, henna, lemongrass, marjoram, and thyme at various concentrations were tested for their effects on the germination of *U. appendiculatus* urediniospores and controlling bean rust in the field. One kilogram of each plant was combined with 1 liter of sterilized distilled water and blended for 5 minutes. The mixture was filtered through double muslin layers, centrifuged for 10 minutes at 3000 rpm, sterilized with a glass filter, and designated as standard solutions (S). Lower concentrations S/2, S/4 and S/8 were prepared by diluting the standard solution with sterilized distilled water (Nisa *et al*., 2010).

2.2 The fungicide

The recommended dose of the fungicide Domark 10 EC (10-12.5% tetraconazole) was applied at 0.5 ml/L, following the Approved Recommendations for Agricultural Pest Control, Ministry of Agriculture, Egypt to evaluate its effectiveness in inhibiting the germination of *U. appendiculatus* urediniospores and controlling bean rust in the field, compared to the plant extracts.

2.3 Source of the uridinespores

Bean plants in several locations at Khatatba, Menoufia Governorate, Egypt were identified as infected plants according to typical rust symptomes and identification of *U. appendiculatus* uridinespores (Chung *et al*., 2004). Spores were scraped from infected leaves by camel hair brush, gathering and keeping on aluminum foil at 4°C until needed (El-Fawy *et al.*, 2021).

2.4 In vitro evaluation against urediniospores germination

Spore suspension was prepared in sterilized distilled water and adjusted to a concentration of 1x10^6 spores/L using a hemocytometer. On a sterile hollow slide, 50 µl of the spore suspension (approximately 50 spores) and 0.5 ml of a 5% sucrose solution were added, followed by 0.5 ml of plant extract concentrations (S/2, S/4, and S/8), Domark 10 EC, or sterilized distilled water as a control. Each treatment was replicated three times. The slides were stored in a closed, sterile Petri dish lined with moist filter paper and incubated at 25±2°C for 72 hours (Zyton and Ahmed, 2016). A light microscope was used to count germinating urediniospores with visible germ tubes. Germination percentage was recorded, and inhibition of urediniospore germination was calculated using the following equation:

$$\text{Inhibition \%} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

2.5 In vivo experiments

Field studies were conducted during the 2022-23 and 2023-24 growing seasons in naturally occurring *U. appendiculatus*-infected environments in Khatatba, Menoufia governorate, Egypt. A randomized block design was used with three replicate plots for each treatment, each measuring 10.5 m² (3x3.5 m) and containing three rows. Bean seeds 'Giza 3' were planted in two lines, 15 cm from the irrigation hose, on October 1st. The plant extracts (S/2) and Domark 10 EC at 0.5 ml/L were applied to the plants weekly starting two weeks after planting until three weeks before harvest. A surfactant and sticker material were included at a rate of 0.5/L. All recommended cultural practices for bean production were followed.

2.5.1 Disease assessment

After the last spry, disease incidence was measured by expressing the number of infected plants as a proportion of the total number of examined plants. Also, the disease severity was estimate based on the scale (1-5) developed by Stavely (1985).

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of cultivated plants in plot}} \times 100$$

$$\text{Disease severity} = \frac{\Sigma\,(\text{n} \times \text{v})}{6N} \times 100$$

$$\text{Treatment efficacy} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

Where: n = Number of leaves in each category, v = Numerical value of each category, and N = Total number of leaves in the sample.

2.5.2 Bean yield

At harvest, the number and weight of pods per plant were counted, and bean yield for each treatment was weighed and expressed in kg per feddan (feddan = 4200 m² = 0.420 hectares = 1.037 acres).

$$Efficacy = \frac{Treatment - Control}{Control} \times 100$$

2.6 Oxidative enzymes and chemical components

The activities of peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase were assessed in leaves collected after the last spray. Meanwhile, total carbohydrates, total nitrogen, and total phenols were measured in bean pods at harvest (AOAC, 2005).

2.7 Statistical analysis

The one-way ANOVA, as described by Gomez and Gomez (1984), was utilized to analyze the collected data. Treatment means were compared using the least significant difference (LSD) at a 5% probability level.

3. Results

3.1 In vitro evaluation against urediniospores germination

The data presented in Table (1) indicates that extracts from basil, henna, lemongrass, marjoram, and thyme significantly inhibit the germination of *U. appendiculatus* urediniospores *in vitro* at the three concentrations (S/2, S/4, and S/8) compared to the fungicide Domark 10 EC, which served as a reference. All treatments resulted in a marked reduction in germination compared to the control, with inhibition increasing as the concentration was raised. Among the treatments, Domark 10 EC proved to be the most effective followed by lemongrass and marjoram extracts at S/2 concentration which led to 7.30%, 21.70%, and 27.3% germination rates, along with inhibitions of 91.9%, 76.0%, and 69.8%, respectively, when compared to the control treatment.

Table (1): Effects of five plant extracts and the fungicide Domark on the germination of *U. appendiculatus* urediniospores after 72 hours incubation at 25±2°C.

Treatment	Concentration*	Germination (%)	Inhibition (%)
Basil	S/2	38.2	57.8
	S/4	45.0	50.3
	S/8	56.7	37.3
Henna	S/2	31.4	65.3
	S/4	35.0	61.3
	S/8	46.0	49.2
Lemon grass	S/2	21.7	76.0
	S/4	27.9	69.2
	S/8	33.5	63.0
Marjoram	S/2	27.3	69.8
	S/4	36.0	60.2
	S/8	41.2	54.5
Thyme	S/2	35.8	60.4
	S/4	40.0	55.8
	S/8	49.7	45.1
Domark 10 EC	500 ppm	7.3	91.9
Control (water)	-	90.5	0.0
LSD at 5%		2.5	2.7

*One kilogram extracted in 1 liter of sterilized distilled water (S), and diluted as S/2, S/4 and S/8..

3.2 The effects of several treatments on the in vivo conditions of bean rust throughout the 2022/23 and 2023/24 seasons

3.2.1 Disease incidence and severity

Regarding disease incidence and severity, the data presented in Table (2) shows that the water extracts of basil, henna, lemongrass, marjoram, thyme, and the fungicide Domark 10 EC significantly reduced the incidence and severity of bean rust during the 2022-2023 and 2023-2024 seasons compared to the control. Domark 10 EC was the most effective treatment, showing reductions of 83.63% and 86.85%. Lemongrass extract followed closely with reductions of 79.05% and 79.19%, while basil extract was the least effective treatment, with reductions of 65.97% and 67.61% in managing the disease.

Table (2): The effect of foliar spraying of five plant extracts and the fungicide Domark on disease frequency and severity of bean rust under field conditions during 2022-2023 and 2023-2024 growing seasons at Khatatba, Menoufia governorate, Egypt.

Treatment	Concentration	Disease frequency (%)				Disease severity (%)			
		2022/23	2023/24	Mean	Efficacy	2022/23	2023/24	Mean	Efficacy
Basil	S*/2	20.25	21.33	20.79	65.97	9.9	10.8	10.35	67.61
Henna		16.23	17.25	16.74	72.60	8.2	9.1	8.65	72.93
lemon grass		12.40	13.20	12.80	79.05	6.1	7.2	6.65	79.19
Marjoram		14.23	15.16	14.70	75.95	7.5	8.3	7.90	75.27
Thyme		18.44	19.11	18.78	69.27	10.2	11.6	10.90	65.88
Domark 10 EC	0.5ml/L	9.80	10.20	10.00	83.63	3.8	4.6	4.20	86.85
Control (water)		60.50	61.70	61.10	0.00	31.4	32.5	31.95	0.00
LSD at 0.05		1.44	1.45	-	-	0.20	0.21	-	-

* One kg extracted in 1L of sterilized distilled water (S) and diluted for use as S/2. Plants sprayed weekly starting two weeks after planting until three weeks before harvest.

3.2.2 Bean yield

The data presented in Table (3) demonstrate that all treatments significantly improved the performance of the 'Giza 3' bean variety regarding yield characteristics. These characteristics include the number of pods per plant, average pod weight per plant (in grams), and yield (in kilograms per feddan) when compared to untreated plants. Extracts of lemongrass, henna, marjoram, and thyme were found to be more effective than Domark 10 EC in enhancing productivity, despite the pesticide's greater ability to suppress urediniospores and reduce the severity of infection. Lemongrass extract yielded the best results with an average of 45.60 and 46.10 pods per plant, 189.9 and 191.20 grams of pod weights, and bean yield of 5472 and 5532 kg per feddan in the two growing seasons, respectively. Marjoram extract ranked second among the extracts for enhancing yield components. In contrast, basil demonstrated the lowest efficacy compared to the other treatments.

Table (3): Impact of foliar spraying of five plant extracts and the fungicide Domark on bean yield under natural rust infection conditions during the 2022-2023 and 2023-2024 growing seasons at Khatatba, Menoufia governorate, Egypt.

Treatments	Concentration	2022-2023 growing season			2023-2024 growing season		
		Pods/plant	Weight of pods (g/plant)	The yield (Kg/feddan)	Pods/plant	Weight of pods (g/plant)	The yield (Kg/feddan)
Basil	S*/2	33.3	163.4	3996	35.0	165.8	4200
Henna		40.8	173.6	4896	41.4	175.5	4968
Lemongrass		45.6	189.9	5472	46.1	191.2	5532
Marjoram		43.4	184.7	5208	44.3	185.3	5316
Thyme		37.9	179.8	4548	38.5	180.4	4620
Domark 10 EC	0.5ml/L	35.7	166.3	4284	36.7	167.6	4404
Control (water)		20.1	101.2	2412	21.2	102.1	2544
LSD at 5%		3.5	4.1	5.6	3.7	4.5	5.1

* One kg extracted in 1L of sterilized distilled water (S) and diluted for use as S/2. Plants sprayed weekly starting two weeks after planting until three weeks before harvest.

3.3 Chemical components

The findings presented in Table (4) indicate that the application of various plant extracts or the fungicide Domark 10 EC significantly increased the total carbohydrates, protein, and phenol content in the treated bean plants across both growth seasons of 2022-2023 and 2023-2024 when compared to untreated plants. Among the treatments, lemongrass showed the most pronounced effects, with notable increases in total carbohydrates (4.98 and 5.10 mg/g dry weight), protein (19.22% and 19.54%), and phenols (7.88 and 8.02 mg/100g fresh weight), followed by marjoram. Conversely, basil extracts had the least impact on the chemical composition when compared to the other treatments, including the untreated bean plants.

Table (4): Impact of foliar spraying of five plant extracts* and the fungicide Domark on chemical compounds under natural rust infection conditions during the 2022-2023 and 2023-2024 growing seasons at Khatatba, Menoufia governorate, Egypt.

Treatments	Concentration	2022-2023 growing season			2023-2024 growing season		
		Total carbohydrates (mg/g dry Weight)	Total protein (%)	Total phenols (mg/100g FW)	Total carbohydrates (mg/g dry Weight)	Total protein (%)	Total phenols (mg/100gFW)
Basil	S*/2	3.49	15.94	5.24	3.56	16.11	5.37
Henna		4.55	17.93	6.89	4.75	18.22	7.11
Lemon grass		4.98	19.22	7.88	5.10	19.54	8.02
Marjoram		4.83	18.16	7.25	4.87	18.35	7.33
Thyme		4.12	17.19	6.22	4.21	17.48	6.45
Domark 10 EC	0.5ml/L	3.64	16.48	5.97	3.88	17.56	6.14
Control (water)		2.10	11.97	2.60	2.12	12.23	2.68
LSD at 5%		0.10	1.26	0.12	0.11	1.28	0.13

* One kg extracted in 1L of sterilized distilled water (S) and diluted for use as S/2. Plants sprayed weekly starting two weeks after planting until three weeks before harvest.

3.4 The way that enzymes respond to the emergence of rust disease and bean plant resistance

The data presented in Table (5) indicates that the application of plant extracts or the fungicide Domark 10 EC during the 2022-2023 and 2023-2024 growing seasons significantly enhanced the activity of peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase enzymes in bean plants compared to untreated plants. Among the treatments, lemongrass extract proved to be the most effective, resulting in increases of 0.383 and 0.393 unit/mg protein for peroxidase, 0.271 and 0.273 unit/mg protein for polyphenol oxidase, and 0.493 and 0.497 unit/mg protein for phenylalanine ammonia lyase. Conversely, basil extract was the least effective treatment.

Table (5): Impact of foliar spraying of five plant extracts* and the fungicide Domark on oxidative enzyme under natural rust infection conditions during the 2022-2023 and 2023-2024 growing seasons at Khatatba, Menoufia governorate, Egypt.

Treatments	Concentration	Peroxidase activity (unit/mg protein)		Polyphenoloxidase (unit/mg protein)		Phenylalanine ammonia lyase (unit/mg protein)	
		2022-2023	2023-2024	2022-2023	2023-2024	2022-2023	2023-2024
Basil	S*/2	0.344	0.350	0.199	0.208	0.389	0.399
Henna		0.371	0.374	0.245	0.251	0.477	0.481
lemon grass		0.383	0.392	0.271	0.273	0.493	0.497
Marjoram		0.378	0.381	0.263	0.265	0.485	0.498
Thyme		0.369	0.388	0.227	0.236	0.428	0.443
Domark 10 EC	0.5ml/L	0.355	0.367	0.208	0.222	0.401	0.422
Control (water)		0.171	0.173	0.113	0.114	0.125	0.128
LSD at 0.05		0.09	0.11	0.12	0.14	0.23	0.25

* One kg extracted in 1L of sterilized distilled water (S) and diluted for use as S/2. Plants sprayed weekly starting two weeks after planting until three weeks before harvest.

4. Discussion

Researchers have conducted several studies and trials demonstrating the value of plant extracts and oils as eco-friendly and effective agents in protecting against diseases, particularly bean rust, while also enhancing productivity. Aromatic plant extracts such as henna, lemongrass, marjoram, thyme, and basil were tested as eco-friendly alternatives to the fungicide Domark 10 EC, which served as a reference. Both the plant extracts and the fungicide inhibited the *in vitro* germination of urediniospores and reduced the incidence and severity of *U. appendiculatus* on bean plants. The main active constituents of basil essential oil that effectively manage bean rust disease are mono-terpenes and alcohol (Adjou-Euloge *et al*., 2012). According to Zyton and Ahmed (2016), essential oils from plant extracts may have a preventive effect against rust due to their toxic components. This research suggests that additional chemicals found in oils, even in lesser concentrations, may indirectly contribute to disease control by stimulating plant defense responses. The results align with findings from Ismail and Afifi (2019) and Abo-Elyousr *et al*. (2021), which highlighted that both biotic and abiotic factors influence the germination of *U. appendiculatus* urediniospores *in vitro* and *in vivo*. The triazole-type fungicide works by disrupting the cell membrane assembly of pathogenic fungi through lipid synthesis, inhibiting fungal ergosterol production, and preventing acetyl-CoA from being converted to steranase molecules, as reported by Zyton and Hassan (2017). Plant essential oils are considered significant sources of biologically active compounds with antibacterial, fungicidal, insecticidal, and nematocidal properties. They can indirectly inhibit infections by altering the surrounding environment, promoting plant growth, and enhancing antibiosis and defensive mechanisms in plants (Sousa *et al*., 2021). Their highwater solubility allows for rapid movement within the sap circulation, while their lipid solubility ensures swift absorption through the plant's tissue. Additionally, the substance is absorbed into the plant after spraying, protecting it from the inside out (Basiony *et al*., 2023). The outcomes of the experiments can be understood in terms of the chemical effects of antioxidants (Ahmed and Shaheen, 2016) and biotic factors that produce growth regulators (Abo-Elyousr *et al*., 2021). Antioxidants are known to enhance plant physiology and metabolism and induce systemic resistance (Ahmed *et al*., 2023). To combat bean rust, plant essential oils operate through various mechanisms, including breaking down cell walls, damaging the cytoplasmic membrane and its proteins, and increasing permeability, which allows cell contents to leak out (El Fawy *et al*., 2023). In terms of yield metrics such as the number of pods per plant, pod weight per plant, and overall yield, all treatments significantly improved bean performance compared to untreated plants. Bean plants treated with extracts, including Kocide DF, nettle, pawpaw, and neem, produced higher yields. This indicates that these treatments have strong suppressive effects against bean rust severity and occurrence, due to either the active chemicals present, breakdown products, or both. This has an indirect influence on plant performance and production (Monda *et al*., 2009). The extent of improvement in these parameters varied depending on the measurement, the severity of bean rust, and the type of plant extract used for treatment (Ahmed and Shaheen, 2016). Many studies have reported similar findings regarding the management of bean rust and increased productivity. For instance, Elkhwaga *et al*. (2018) found that both water and methanol extracts from tested plants such as henna, acalypha, chinaberry, pomegranate, and lantana effectively prevented the germination of spores from, the causative agent of wheat leaf rust disease, in both *in vitro* and *in vivo* settings. Additionally, these extracts improved various components of wheat yield. Numerous active antimicrobial compounds found in medicinal plants have beneficial effects and are commonly used to treat various diseases while enhancing yield characteristics (Amine, 2019; Basiony *et al*., 2023). Applying plant extracts or the fungicide Domark 10 EC significantly increased the levels of total carbohydrates, protein, phenols, and the activities of enzymes such as peroxidase (PO), polyphenol oxidase (PPO), and phenylalanine ammonia lyase (PAL). The effectiveness of these enzymes and plant extracts in activating various biochemical defense responses against bean rust was assessed using GC-MS, which detected numerous physiologically active components including fatty acids, esters, alcohols, phenols, antioxidants, carotenoids, enzymes, and steroids (El Fawy *et al*., 2023). Polyphenol oxidase (PPO), an enzyme containing copper, catalyzes the oxidation of phenolic compounds into quinones. Its role in disease resistance is associated with its function in the final oxidation of damaged plant tissue. PAL is the primary enzyme in the flavonoid and phenyl-propanoid pathways, while PO has multiple roles in plant defense (Reddy *et al*., 2014). Elevated levels of PAL, PO, and PPO have been identified as factors contributing to reductions in disease severity (Hancock *et al*., 2015). Furthermore, Zyton and Ahmed (2016) demonstrated that a functioning phenol oxidase system is essential for utilizing endogenous phenolic compounds in plant disease resistance. Inducing systemic resistance is a novel strategy for protecting plants from infections and is much less harmful to the environment and plant products than relying on toxic agrochemicals for disease management (Zyton and Ahmed, 2016). As noted by Prasannath (2017), PO converts phenolics to quinones, which inhibit fungal growth and spore germination, and also plays a role in lignin production. The results of these studies support earlier findings indicating that the accumulation of phenolic compounds, defense-related enzymes, pathogenesis-relatedroteins, and systemic acquired resistance suggests that treating plants with chemicals and biotic inducers may effectively help manage plant diseases (Omara *et al*., 2022).

5. Conclusion

Three concentrations of henna, lemon, marjoram, thyme, and basil water extract were evaluated as alternatives to chemical fungicides for controlling bean rust *in vivo* and *in vitro* alongside the fungicide Domark 10 EC. All treatments significantly reduced the germination percentage of *Uromyces appendiculatus* urediniospores *in vitro*. In field trials during the 2022-23 and 2023-24 seasons in Khatatba, Menoufia Governorate, Egypt, all treatments decreased the incidence and severity of bean rust. They also increased bean yield, pod count, and weight per plant. Treated plants showed elevated levels of total carbohydrates, proteins, phenols, and enzyme activities, including peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase, compared to untreated plants. Lemongrass extract was the most effective, followed by marjoram, while basil was the least effective over the two seasons.

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